WHEAT ENDOSPERM COMPRESSIVE STRENGTH PROPERTIES AS AFFECTED BY MOISTURE

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ABSTRACT. *Tempering, the addition of water to wheat before milling, is routinely performed to toughen the pericarp of the kernel and thus improve the efficiency of flour extraction. In an effort to better understand how tempering affects the kernel's internal structure, the effect of moisture on the physical strength properties of wheat kernel endosperm on an in situ basis was studied. Five soft and five hard wheat samples, available from the U.S. National Institute of Standards and Technology's Standard Reference Materials Program for the purpose of standardizing wheat hardness instruments, were examined. Geometrically precise cylinders of wheat endosperm (1 mm diameter $\times$ 3 mm height) were conditioned under one of five relative humidities (~0 to 93% RH, producing moisture contents of 3 to 28%, dry basis), then tested for the following compressive strength properties: maximum compressive stress (S_{max}), modulus of elasticity (E), work to maximum stress [$W(S_{max})$], and strain at maximum stress [$e(S_{max})$]. Large variations in strength properties occurred within the same sample and humidity, as seen by coefficients of variation reaching as high as 56%. Mean values of S_{max}, E, $W(S_{max})$, and $e(S_{max})$, based on 8 to 13 endosperm cylinders per sample $\times$ humidity setting, were normalized to a reference moisture content of 13% d.b. Normalized values of the first three properties were linearly fitted to moisture content and statistically tested to determine the similarity in response among samples. S_{max} was most linearly related to moisture content. When samples within a hardness class were pooled, the slopes of the normalized S_{max} regression equations were significantly different ($P < 0.01$) for hard and soft wheats. A similar pooling procedure for normalized E revealed a moderately significant ($P < 0.05$) difference between slopes of soft and hard classes. For $W(S_{max})$, most samples did not exhibit a sensitivity to moisture; however, when pooled into soft and hard classes, the difference between these classes was highly significant ($P < 0.01$). The response of $e(S_{max})$ to moisture was constant except at the highest moisture content (24 to 28% d.b.), where the values were approximately twice as large.*

Keywords. *Wheat, Physical properties, Moisture, Hardness, Stress, Strain.*

Wheat hardness, a physical property that is a manifestation of the biochemical interaction between the endosperm storage proteins and starch granules (Barlow et al., 1973; Greenwell and Schofield, 1986; Jolly et al., 1993; Greenblatt et al., 1995), is important in the determination of milling throughput, equipment design, and energy requirements. It is equally important to the baking and processing industries, which rely upon the differences in textural properties of hard and soft wheats when forming various food products. Numerous studies have dealt with efforts to standardize the measurement of wheat hardness, including visual inspection of crushed endosperm (Mattern, 1988), near-infrared reflectance (NIR) of ground meal (Williams and Sobering,1986; Norris et al., 1989), force of slicing (Eckhoff et al., 1988), force and energy of crushing (Lai et al., 1985; Pomeranz et al., 1988; Martin et al., 1993), and acoustical properties of a kernel during grinding (Massie et al., 1993). Knowledge of the physical strength properties of wheat endosperm is important because these properties are directly related to wheat hardness. Fundamental studies on single kernel physical strength have been performed on intact kernels with simplified geometry (Arnold and Roberts, 1969), end-faced kernels with cross-sections defined by digital image analysis (Kang et al., 1995), end-faced kernels of one cultivar subjected to crack propagation during loading-unloading cycles (Dobraszczyk, 1994), and machined cylinders of wheat endosperm (Glenn et al., 1991). Stemming from the commercial manufacture of a single kernel characterization system (SKCS) initially developed by Martin et al. (1993), the Standard Reference Materials Program of the U.S. National Institute of Standards and Technology (NIST), in cooperation with the USDA Federal Grain Inspection Service Program, has made available for purchase, a set of ten wheat samples (RM 8441) of specified hardness. The hardness of these samples has been characterized by the empirical scales used in NIR and in SKCS measurements for the purpose of allowing users the ability to standardize these instruments. The furnished values are based on normal ambient storage conditions (23°C, 11 to 13% d.b.) and therefore do not provide an indication of the sensitivity of hardness to moisture content or humidity level.

Tempering, which is the addition of water to wheat before milling, is a routine procedure that enhances the efficiency of flour extraction. Its purpose is essentially to

Article was submitted for publication in May 1999; reviewed and approved for publication by the Food & Process Engineering Institute of ASAE in February 2000. Presented as ASAE Paper No. 99-6051.

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toughen the pericarp to enhance the separation of the endosperm. Hardness is known to be indicative of the rate and quantity of water uptake during the tempering procedure, and although it is generally accepted that hard wheat endosperm diffuses water at a slower rate than soft wheat endosperm, the exact nature of the interaction is not well understood but appears to be affected by vitreousness and the agglomeration of starch and proteins within the endosperm (Pomeranz and Williams, 1990). Milling studies have traditionally been directed toward bulk samples of grain, with a much smaller portion of studies on single kernels, and an even smaller portion focusing on moisture within the kernel.

Although studies have been conducted on the effect of moisture on NIR hardness (Windham et al., 1993; Gaines and Windham, 1998), the effects on the physical strength properties of the endosperm are less prevalent. Eckhoff et al. (1988) found small but significant differences in peak force of both soft and hard wheats caused by differences in moisture content at levels of 9, 11, and 13%. Given that an interaction between moisture content and cultivar was significant, a recommendation was made that additional testing was needed to examine the extent of this interaction. Working with five wheat varieties at three levels of moisture (10, 14, and 18% d.b.), Kang et al. (1995) found that the slope of the stress-strain compression curve before the yield point, which they termed the modulus of deformability, decreased with increase in moisture, as did the stress and strain at yield point. In perhaps the most extensive work on the *in-situ* measurement of physical strength properties of wheat endosperm, Glenn et al. (1991) reported on the compressive and tensile stress properties of geometrically precise cylinders for a set of common soft and hard wheats. Included in their study was an examination of the effect of moisture (2 to 30% d.b.) on compressive strength, involving two soft and three hard varieties. The present study has adopted the wheat cylinder machining procedure of Glenn et al. (1991) to further examine compressive strength under varying levels of moisture. The study's objectives were to characterize the effect of moisture on wheat endosperm compressive strength for the 10 NIST SRM samples and relate the physical strength properties to the current definitions of wheat hardness.

Materials and Methods

Wheat

Ten samples of wheat were obtained from NIST (Gaithersburg, Md.) as Reference Material (SRM) No. 8441 (table 1). These standard samples, consisting of five soft wheats and five hard wheats, are used for the purpose of standardizing near-infrared spectrophotometers to an empirical hardness scale. Likewise, this same set is also used to standardize the commercial models of the SKCS (Perten Instruments, Springfield, Ill.), which utilizes force of crush data to measure hardness on the same scale. Two additional samples, which were soft wheats (cvs. Penawawa and Vanna), were also analyzed for the purpose of verifying strength versus moisture relationships developed from the NIST samples.

Table 1. Wheat samples examined in study

Sample	Variety	Wheat Class*	NIR Hardness†	SKCS Hardness‡
NIST Soft No. 1	Cardinal	SRW	30.0	24.7
NIST Soft No. 2	Titan	SRW	29.9	26.1
NIST Soft No. 3	Madsen	SWW	31.1	34.4
NIST Soft No. 4	Malcom	SWW	29.8	34.2
NIST Soft No. 5	Tres	Club	31.5	36.6
NIST Hard No. 1	TAM105	HRW	74.7	79.0
NIST Hard No. 2	Arapahoe	HRW	75.8	66.3
NIST Hard No. 3	Newton	HRW	63.7	68.5
NIST Hard No. 4	Yecora Rojo	HRS	77.5	63.5
NIST Hard No. 5	Len	HRS	91.8	85.5
Penawawa	Penawawa	SWS	n/a	n/a
Vanna	Vanna	SWS	n/a	n/a

* SRW = soft red winter, SWW = soft white winter, HRW = hard red winter, HRS = hard red spring, SWS = soft white spring.

† Near-infrared hardness. Measured according to American Association of Cereal Chemists' Approved Method 39-70. Values furnished in NIST Report of Investigation: Reference Material 8441: Wheat Hardness (5 December 1997).

‡ Single Kernel Characterization System hardness. Measured on SKCS 4100 instrument (Perten Instruments of North America, Springfield, Ill.) and furnished in NIST Report of Investigation: Reference Material 8441: Wheat Hardness (5 December 1997).

Procedure

Wheat endosperm cylinders were machined following the procedure of Glenn et al. (1991). Essentially, each kernel was candled to ensure it was free of internal cracks, then filed on its germ end until a flat surface, nominally perpendicular to the long axis, was created just beyond the depth of the germ. Cyanoacrylate glue (Krazy® Glue, wood formula) was applied to the filed surface and the kernel was mounted on an aluminum pedestal (11 mm diameter × 4 mm thickness × 6 mm shaft diameter) with an attempt to position the kernel so that the centroid of one cheek region was centered on the pedestal face. The kernel was allowed to set overnight before the brush end of the kernel was faced by placing the kernel in a vertical milling machine and revolving the kernel against a stationary file to a finished length of 3.5 mm. The cylindrical shape was obtained by placing the kernel in a small machinist's lathe (Compact 5, Emco, Hallein, Austria) and turning the kernel in one pass to a 1.0 mm diameter and 3.0 mm length, leaving the 0.5-mm-thick base for torsional strength during the turning procedure (fig. 1).

Each cylinder was inspected for internal fissures and the inclusion of the crease. Only kernels with no crease or a trace of the pericarp from the crease were eventually accepted for strength properties analysis, though cylinders possessing larger portions of the crease were in many cases tested in compression. Cylinders of a given sample and assigned humidity level were prepared as a group of 12 replicates. Each group was placed in one of five controlled humidity environments (desiccant or saturated salt solution, 25°C), corresponding to 0 (Molecular Sieve), 22 (potassium acetate), 53 (magnesium nitrate), 75 (sodium chloride), and 93% (potassium nitrate) RH (Greenspan, 1977). Cylinders were allowed to equilibrate a minimum of 5 d and remained in their humidity environment until immediately before strength testing.

Compressive strength tests were performed on a universal testing machine (model 4464, Instron Corp., Canton, Mass.) equipped with a 500-N load cell. The

Figure 1–Faced and turned wheat endosperm cylinder (1.0 mm diameter × 3.0 mm length) before compression testing.

pedestal containing each endosperm cylinder was inserted in the chuck of the crosshead, with the stationary surface consisting of a polished aluminum block. Crosshead speed was set low (0.5 mm/min), in keeping with Glenn et al. (1991), who found that variability, as gauged by repeated measures of compressive strength, increased with increase in crosshead speed (0.5 to 500 mm/min range examined). The sampling rate was 6.67 points/s. Force and displacement readings were collected well beyond the point of maximum force, which occurred between 0.046 and 0.45 mm. Depending on the force vs. displacement data, collection ceased either soon after the occurrence of maximum force or at the predefined limit of 1.0 mm.

The following physical strength properties were calculated using the Instron software (ver. 7.34.00): maximum stress (S_{max}, Function **1.4**, MPa), modulus of elasticity (E, **19.3**, MPa), work to maximum stress [$W(S_{max})$, **43.1**, MJ/m^3], and strain at maximum stress [$e(S_{max})$, **1.6**, %]. E was determined by (1) automatically subdividing the stress versus strain curve into six equal contiguous regions between the start of the test and the point of maximum stress; (2) selecting the pair of adjacent regions whose sum of slopes, as determined by a least squares fit line for each region, was greatest; and (3) selecting the greater of the two slopes from that pair. Further, E and $e(S_{max})$ were based on the original 3.0-mm specimen length, which did not include the 0.5-mm base thickness. The omission of the base, which could directly affect the values of $e(S_{max})$ and E, and theoretically affect compressive strength and work input, was deemed necessary for two reasons: the base absorbed some of the cyanoacrylate glue, causing the base to be much harder than the native endosperm; secondly, the cross-sectional shape of the base was comparatively irregular, thus making it difficult to define, as well as restrictive to lateral expansion.

Moisture Content Determination

Twenty grams of each wheat sample were pearled (Strong-Scott, Seedburo Equipment Co., Chicago, Ill.) 40 to 105 s (depending on hardness) until nearly all pericarp and germ were removed, leaving approximately 11 g of intact endosperm. The pearled material was split into five portions of equal mass, whereupon each portion was equilibrated to one of the five humidities for eight days. Moisture contents were performed gravimetrically by convection oven drying (130°C) for 2 h and reported on a dry basis (ISO, 1985).

Analysis

For each sample, the mean value of a given physical strength property at each moisture level was determined. First-order linear regression equations, with mean physical strength property as the dependent variable and moisture content as the independent variable, were developed for S_{max}, E, and $W(S_{max})$. Mean physical strength property values were subsequently normalized by dividing them by their corresponding predicted value at 13% d.b. This moisture content was chosen as a reference level because it represents the state of wheat under typical storage conditions. Thus, each normalized mean value of S_{max}, E, or $W(S_{max})$ is a dimensionless quantity that represents the decimal change in physical strength property when the moisture condition deviates from a nominally acceptable storage level (13% d.b.) to that considered either dryer (3% and 8 to 9% d.b.) or wetter (16 to 17% and 25 to 28% d.b.) than normal. A first-order linear regression was performed on each NIST sample's five normalized means. Using the Proc Mixed procedure of SAS (Littell et al., 1996), orthogonal contrasts within an analysis of covariance were performed to determine whether the slope of one sample's regression line was equal to that of another sample's line.

Normalization of the mean values for $e(S_{max})$ was performed in a similar manner to that of the other strength properties, with one exception. Because of the near constancy in mean value of $e(S_{max})$ at the four lower moistures (see Results), only these moisture levels were used in the initial regression to determine the reference value at 13% d.b. Curve fitting was not subsequently performed on the normalized $e(S_{max})$ values, owing to a clearly nonlinear relationship between this property and moisture.

Results

Cylinder failure patterns varied widely, largely depending on moisture content. Kernels equilibrated in the 0 and 22% RH environments (moisture < 9% d.b.) often produced a brittle, audible, 45° shear-plane fracture (fig. 2), while those at the highest humidity (93% RH, moisture > 24% d.b.) demonstrated shallow and broad force-displacement behavior and a failure pattern (not shown) that was characterized by a crumbling of the entire cylinder or a crumbling localized to the cylinder's free end. Typical stress versus strain curves for cylinders equilibrated to the five humidities are shown for soft (NIST Soft No. 2) and hard (NIST Hard No. 5) wheats (fig. 3). As seen by this example, maximum stress occurred between 3 and 9% strain with exception of the cylinders at the highest moisture, which exhibited a greater degree of non-linear stress versus strain behavior. At the 24 to 28% d.b. level,

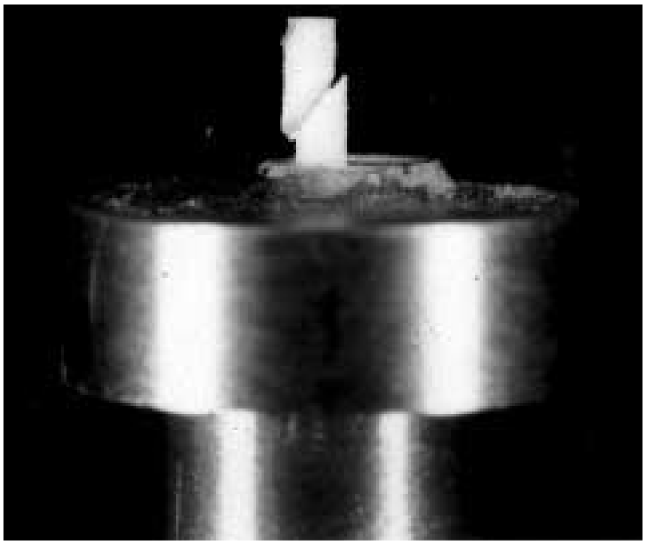
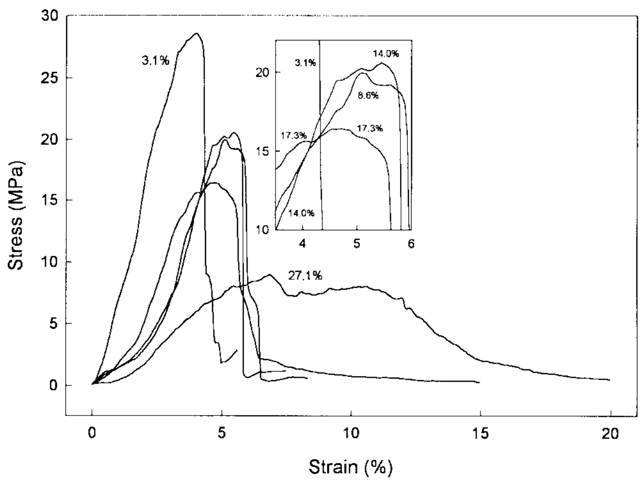
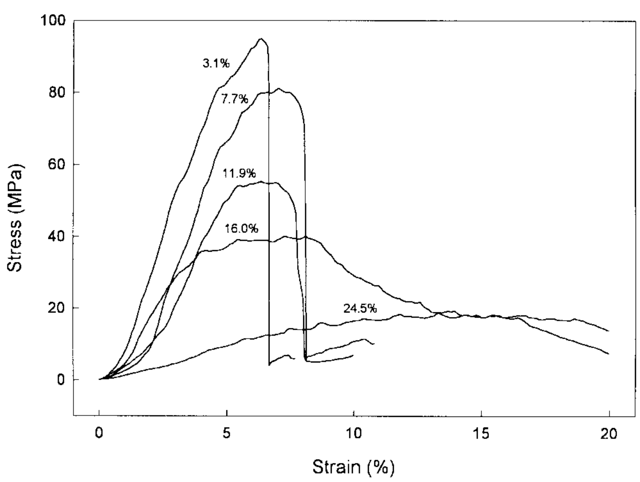


Figure 2–Example of a low moisture specimen after compressive failure.



(a)



(b)

Figure 3–Stress versus strain behavior for wheat endosperm cylinders under compressive loading. Each curve represents the typical behavior of a cylinder equilibrated to one of five humidity levels, with moisture content (d.b.) displayed beside curve (a = NIST Soft No. 2; b = NIST Hard No. 5).

maximum stress was often less than one-half its value at the intermediate moisture content (11 to 14% d.b.), while the accompanying strain was often greater than 10%.

All force deformation curves were visually inspected for atypical characteristics such as an excessively broad and shallow-sloped trace, which would be indicative of the presence of a preexisting internal crack or, at the highest moisture level, failure due to bending caused by the cylinder's axis being no longer perpendicular to its pedestal face. From a total of 627 curves, three were considered subjectively as outliers and removed from further analysis. All remaining curves, ranging from 8 to 12/sample × humidity, were used to determine the mean and standard deviation of each strength property (tables 2 and 3). Kernel-to-kernel variability (i.e., replicate error) in strength property was large, as seen by a range in the coefficient of variation (considering only the NIST samples) as follows: S_{max} was 8.1 to 49.2%; E was 8.3 to 52.0%; $W(S_{max})$ was 17.3 to 56.4%; $e(S_{max})$ was 10.7 to 37.2%. Thus, when analyzing single kernel physical strength properties, it was imperative that measurements were made on many kernels for each treatment. Hard wheats tended to have less kernel-to-kernel variability than soft wheats.

The functional relationship between normalized mean physical strength property and moisture content was partially sample-dependent for S_{max}, while independent of sample for E and $W(S_{max})$ (table 4). Pooling of samples, either within a hardness class (i.e., hard or soft) or across both classes, was performed for the purpose of allowing the

Table 2. Material properties of soft wheat endosperm at five humidification levels*

Sample	Moisture (%, d.b.)	n	S_{max} (MPa)†	E (MPa)‡	$W(S_{max})$ (MJ/m^3)§	$e(S_{max})$ (%)‖
NIST Soft No. 1	3.1	10	29.6 ± 11.1	1040. ± 396	0.643 ± 0.317	3.60 ± 0.59
	8.6	11	26.2 ± 5.0	1080. ± 387	0.467 ± 0.220	3.46 ± 1.29
	13.9	10	18.3 ± 6.3	805. ± 319	0.364 ± 0.132	3.09 ± 0.77
	17.3	10	18.0 ± 4.7	724. ± 235	0.409 ± 0.113	3.36 ± 0.60
	26.9	8	10.4 ± 2.8	207. ± 76	0.415 ± 0.111	7.57 ± 2.21
NIST Soft No. 2	3.1	10	26.8 ± 4.0	1120. ± 239	0.558 ± 0.146	3.14 ± 0.56
	8.6	10	20.4 ± 5.5	1040. ± 491	0.412 ± 0.209	3.09 ± 0.87
	14.0	10	20.9 ± 2.9	968. ± 209	0.421 ± 0.126	3.05 ± 0.57
	17.3	10	16.7 ± 3.7	797. ± 235	0.292 ± 0.083	2.83 ± 0.43
	27.1	10	10.4 ± 1.8	246. ± 91	0.455 ± 0.079	7.38 ± 1.26
NIST Soft No. 3	2.6	10	31.4 ± 15.5	1450. ± 754	0.602 ± 0.340	3.03 ± 0.95
	9.0	10	30.5 ± 10.9	1070. ± 292	0.745 ± 0.337	3.50 ± 0.61
	13.0	11	26.9 ± 6.7	1250. ± 303	0.493 ± 0.226	2.86 ± 0.61
	17.5	11	19.3 ± 5.7	885. ± 250	0.362 ± 0.123	3.02 ± 0.49
	27.6	11	15.4 ± 3.7	400. ± 99	0.536 ± 0.151	6.09 ± 1.17
NIST Soft No. 4	2.9	10	39.6 ± 15.7	1290. ± 533	0.918 ± 0.491	3.70 ± 0.96
	8.7	10	32.4 ± 7.4	1500. ± 350	0.601 ± 0.183	2.90 ± 0.60
	12.5	10	24.2 ± 2.4	1200. ± 146	0.415 ± 0.090	2.72 ± 0.36
	17.4	11	19.8 ± 2.4	846. ± 118	0.391 ± 0.082	3.31 ± 0.41
	27.7	10	12.2 ± 2.9	293. ± 78	0.413 ± 0.151	6.05 ± 0.95
NIST Soft No. 5	2.8	10	33.3 ± 9.7	1500. ± 773	0.710 ± 0.250	3.29 ± 0.77
	9.1	11	25.8 ± 3.6	1190. ± 249	0.446 ± 0.087	2.88 ± 0.42
	13.5	9	24.5 ± 5.0	901. ± 347	0.525 ± 0.108	3.37 ± 0.76
	17.8	11	21.6 ± 6.0	861. ± 194	0.485 ± 0.187	3.38 ± 0.46
	27.9	9	11.9 ± 3.7	275. ± 150	0.445 ± 0.173	6.59 ± 2.04
Penawawa	2.9	11	33.9 ± 8.2	1300. ± 255	0.686 ± 0.248	3.07 ± 0.66
	9.2	9	30.2 ± 4.8	1240. ± 182	0.605 ± 0.144	3.06 ± 0.29
	12.9	11	23.5 ± 3.6	1100. ± 208	0.399 ± 0.118	2.74 ± 0.40
	17.8	12	20.3 ± 2.6	774. ± 134	0.444 ± 0.066	3.46 ± 0.41
	27.6	9	8.9 ± 2.0	159. ± 55	0.466 ± 0.117	8.59 ± 1.94
Vanna	2.6	12	26.2 ± 6.2	1070. ± 260	0.494 ± 0.177	3.01 ± 0.52
	9.3#	12	30.0 ± 15.7	1330. ± 346	0.644 ± 0.737	2.81 ± 0.94
	13.1	12	20.8 ± 3.1	1080. ± 202	0.322 ± 0.058	2.45 ± 0.26
	18.1	11	15.8 ± 2.1	553. ± 79	0.357 ± 0.093	3.60 ± 0.49
	27.9	12	6.3 ± 2.2	116. ± 50	0.262 ± 0.115	8.00 ± 2.27

* Mean ± standard deviation for n endosperm cylinders.
† S_{max} = maximum compressive stress.
‡ E = modulus of elasticity.
§ $W(S_{max})$ = work to point of maximum compressive stress.
‖ $e(S_{max})$ = strain at point of maximum compressive stress.
Excluding one outlying kernel (S_{max} = 77.4 MPa, E = 2090 MPa, $W(S_{max})$ = 2.951 MJ/m^3, $e(S_{max})$ = 5.60%) the means and standard deviations of the material properties are as follows: S_{max} = 25.7 and 5.0; E = 1260 and 262; $W(S_{max})$ = 0.435 and 0.131; $e(S_{max})$ = 2.56 and 0.36.

Table 3. Material properties of hard wheat endosperm at five humidification levels*

Sample	Moisture (%, d.b.)	n	S_{max} (MPa)†	E (MPa)‡	$W(S_{max})$ (MJ/m^3)§	$e(S_{max})$ (%)‖
NIST Hard No. 1	3.1	10	85.8 ± 15.2	2060. ± 274	3.147 ± 1.300	5.93 ± 1.47
	7.9	10	70.3 ± 13.3	2110. ± 322	2.255 ± 0.934	5.28 ± 1.38
	11.5	10	53.1 ± 9.0	1850. ± 247	1.527 ± 0.575	4.33 ± 1.14
	16.3	10	40.5 ± 4.7	1360. ± 234	1.337 ± 0.349	5.13 ± 0.77
	25.0	10	21.1 ± 7.2	538. ± 222	0.908 ± 0.405	6.59 ± 1.35
NIST Hard No. 2	3.0	10	56.0 ± 13.4	1760. ± 449	1.447 ± 0.509	4.18 ± 0.88
	8.6	11	45.5 ± 9.6	1400. ± 335	1.219 ± 0.373	4.32 ± 0.63
	13.3	10	39.5 ± 8.0	1730. ± 475	0.888 ± 0.267	3.53 ± 0.40
	16.9	9	25.4 ± 5.5	1020. ± 258	0.677 ± 0.233	3.96 ± 0.62
	25.2	10	18.5 ± 5.2	400. ± 168	0.926 ± 0.311	8.37 ± 1.69
NIST Hard No. 3	3.2	11	61.4 ± 15.0	1810. ± 321	1.610 ± 0.557	4.14 ± 0.77
	8.2	13	53.4 ± 10.1	1750. ± 289	1.387 ± 0.492	4.11 ± 0.90
	12.5	10	41.2 ± 7.3	1620. ± 335	0.907 ± 0.326	3.36 ± 0.61
	16.0	10	35.4 ± 3.4	1320. ± 261	0.917 ± 0.169	3.94 ± 0.47
	25.0	10	18.3 ± 6.0	371. ± 187	0.864 ± 0.324	8.12 ± 0.88
NIST Hard No. 4	3.0	11	109.3 ± 14.6	2640. ± 349	4.071 ± 1.230	5.92 ± 1.16
	7.4	12	90.5 ± 8.2	2300. ± 267	4.034 ± 1.254	6.61 ± 1.32
	10.7	11	65.7 ± 5.6	2050. ± 170	2.409 ± 0.571	6.24 ± 1.31
	15.7	11	49.3 ± 4.1	1520. ± 193	2.257 ± 0.680	7.08 ± 1.19
	24.6	9	23.8 ± 6.6	391. ± 156	1.527 ± 0.648	12.27 ± 1.31
NIST Hard No. 5	3.1	10	93.4 ± 13.1	2460. ± 278	3.091 ± 1.153	5.28 ± 1.19
	7.7	11	80.9 ± 8.4	2280. ± 369	3.020 ± 0.949	5.76 ± 1.09
	11.9	11	55.4 ± 4.5	1820. ± 296	1.964 ± 0.478	5.82 ± 1.80
	16.0	10	42.4 ± 6.9	1410. ± 296	1.669 ± 0.566	6.14 ± 1.58
	24.5	10	20.9 ± 4.4	359. ± 137	0.912 ± 0.253	9.73 ± 2.73

* Mean ± standard deviation for n endosperm cylinders.
† S_{max} = maximum compressive stress.
‡ E = modulus of elasticity.
§ $W(S_{max})$ = work to point of maximum compressive stress.
‖ $e(S_{max})$ = strain at point of maximum compressive stress.

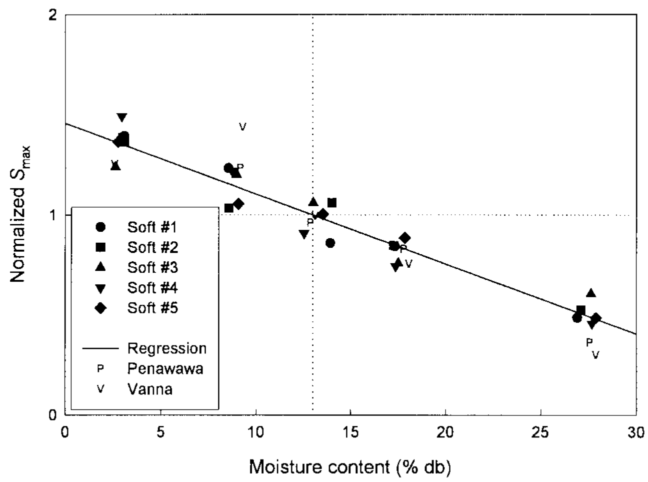
Table 4. Summary of regression analyses: Dependent variable = Normalized mean physical strength property (dimensionless), Independent Variable = Moisture Content (%, d.b.)

NIST Sample	n§	S_{max}* Slope‖	R^2	E† Slope	r^2	$W(S_{max})$‡ Slope	r^2
Soft No. 1	5	–0.0388 abcd	0.970	–0.0457 a	0.908	–0.0182	0.498
Soft No. 2	5	–0.0327 ab	0.941	–0.0420 a	0.902	–0.0099	0.167
Soft No. 3	5	–0.0283 a	0.911	–0.0385 a	0.899	–0.0121	0.200
Soft No. 4	5	–0.0420 bcde	0.955	–0.0436 a	0.844	–0.0332	0.611
Soft No. 5	5	–0.0332 abc	0.977	–0.0474 a	0.983	–0.0155	0.504
Hard No. 1	5	–0.0555 ef	0.979	–0.0478 a	0.921	–0.0538 a	0.866
Hard No. 2	5	–0.0468 cdef	0.957	–0.0467 a	0.787	–0.0262	0.573
Hard No. 3	5	–0.0482 def	0.994	–0.0493 a	0.886	–0.0313	0.740
Hard No. 4	5	–0.0614 f	0.968	–0.0615 a	0.986	–0.0455 a	0.846
Hard No. 5	5	–0.0611 f	0.972	–0.0618 a	0.976	–0.0524 a	0.940
Pooled softs	25	–0.0349 x	0.936	–0.0434 x	0.904	–0.0178 x	0.371
Pooled hards	25	–0.0546 y	0.961	–0.0534 y	0.906	–0.0418 y	0.778
Pooled softs & hards	50	–0.0437	0.907	–0.0478	0.896	–0.0285	0.552

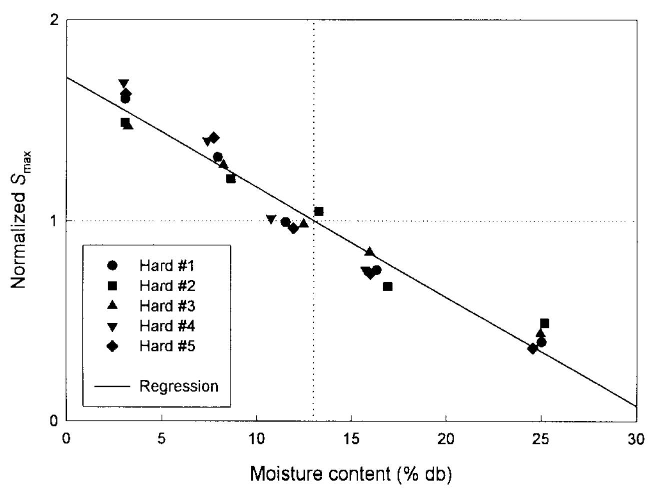
* S_{max} = maximum compressive stress.
† E = modulus of elasticity.
‡ $W(S_{max})$ = work to point of maximum compressive stress.
§ Number of mean values, where each value is the arithmetic mean of 8 to 13 (tables 2 and 3) endosperm cylinders.
‖ Within each slope column, with the five hard and five soft samples as one category and the pooled softs and pooled hards as another category, the same letters following slope values indicate non-significant differences among category members by at least the 5% level. Within these designated categories, absence of a letter indicates a failure to reject hypothesis of zero slope by t-test ($P < 0.05$). Units for slope are 1/(dry basis percentage point).

reporting of general trends. Therefore, it is important to acknowledge that when statistical differences are noted among samples, the general trends obtained from pooled data will have more uncertainty than when pooling is performed with non-statistically different samples. Nevertheless, all pooled results are reported herein because of a belief that such summarizations are directly useful. For S_{max}, the slopes for all 10 NIST samples were significantly different from zero ($P < 0.01\%$), with all being negative in value, ranging from –0.0283 (Soft No. 3) to –0.0614 (Hard No. 4), and r^2 ranging from 0.911 (Soft No. 3) to 0.994 (Hard No. 3). Each of the five soft samples had a slope that was not significantly different ($P < 0.05$) from at least three of the other soft samples. Likewise, each hard sample had a slope that was not significantly different from any of the other hard samples. The slope of the regression line from a pooling of all soft NIST samples (–0.0349, r^2 = 0.936) was significantly different ($P < 0.01$) than the corresponding slope from the pooled hard NIST samples (–0.0546, r^2 = 0.961). Hard wheat was more greatly affected by moisture than soft wheat, as seen by a regression line that was 56% steeper (fig. 4). The normalized maximum stress values for the two non-NIST soft wheat samples (Penawawa and Vanna) were similar to those of the NIST set, which alludes to the generality of the response to moisture.

The slope of each NIST sample's normalized E versus moisture content regression line was not significantly different ($P < 0.05$) from the slopes of any of the other NIST samples' lines (table 4). The degree of correlation, with r^2 ranging from 0.844 (Soft No. 4) to 0.986 (Hard No. 4), was generally lower than that for S_{max}. However, the influence of moisture content on E was more universal. When soft samples were pooled and compared with pooled



(a)



(b)

Figure 4–Effect of moisture content on maximum compressive stress (S_{max}). Each plotted symbol or letter is the mean of 8 to 12 values from individual endosperm cylinders, which is then normalized to a reference moisture content of 13% d.b. The regression line (slope and r^2 values in table 4) arising from the pooling of all five NIST samples within a hardness category is shown on each graph. Graphs and their respective categories are as follows: a = soft wheats, inclusive of five NIST samples and the non-NIST samples, Penawawa and Vanna; b = NIST hard wheats.

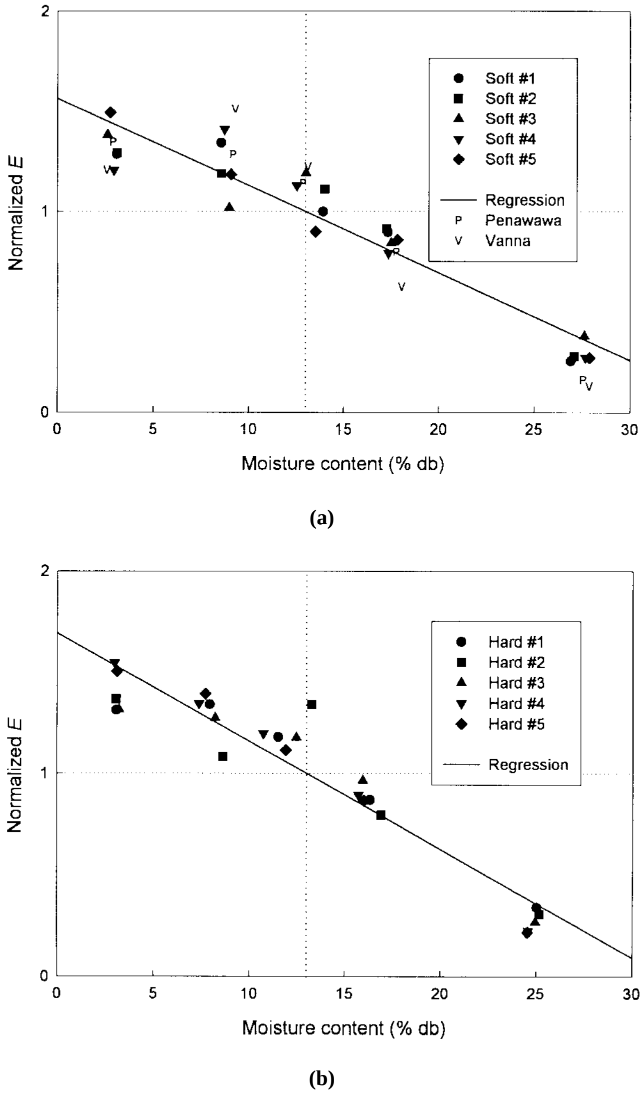


Figure 5–Effect of moisture content on modulus of elasticity (E). (See fig. 4 caption for graph descriptions.)

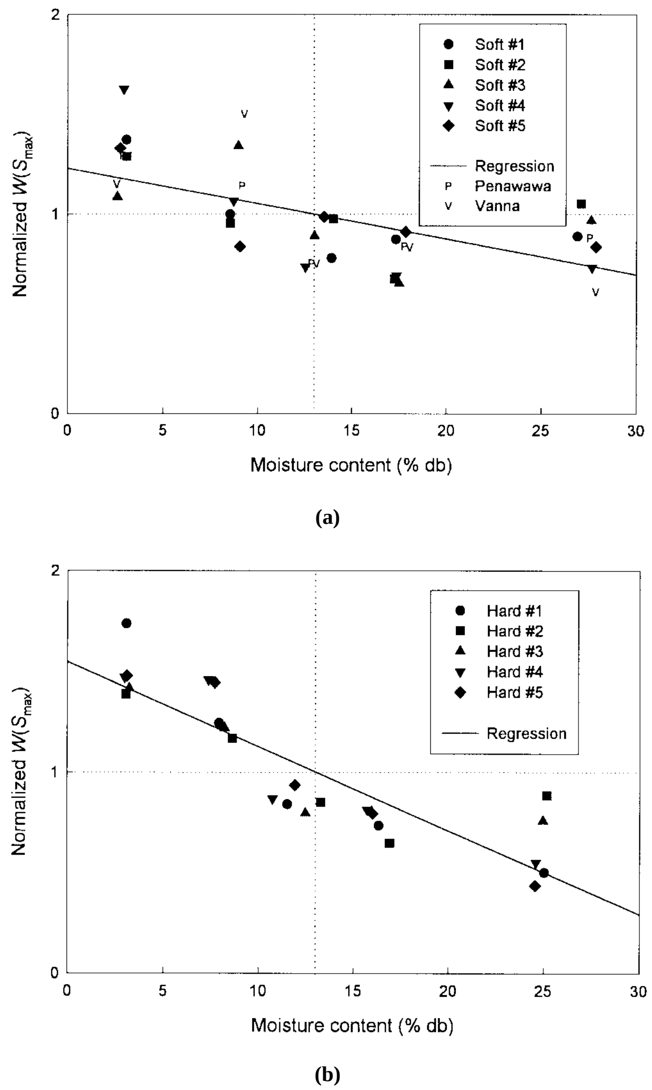


Figure 6–Effect of moisture content on work to point of maximum compressive stress [$W(S_{max})$]. (See fig. 4 caption for graph descriptions.)

hard samples, the slopes of the regression lines were significantly different (–0.0434 vs –0.0534, $P < 0.05$), though their difference is most likely not of practical value (fig. 5). Therefore, a pooling of all soft and hard NIST samples (slope = –0.0478, $r^2 = 0.896$) can be used to generalize the behavior of moisture content on E. As with their values for S_{max}, Penawawa and Vanna demonstrated E values that were similar to those of the NIST set.

For $W(S_{max})$, there was no significant linear relationship with moisture content within any of the NIST soft samples and two of the hard samples (table 4). For the three remaining hard samples, the slopes were not determined to be significantly different from each other. The slope of the pooled NIST soft wheats was significantly different ($P < 0.01$) than that of the pooled NIST hard wheats (fig. 6). In fact, the regression line for the hard wheats (slope = –0.0418, $r^2 = 0.778$) was more than twice as steep as the soft wheat regression line (slope = –0.0178, $r^2 = 0.371$). The response of $e(S_{max})$ to changes in moisture was invariant except at the highest moisture (24 to 28% d.b.) (fig. 7). Without additional data within the 18 to 24% moisture range, it is not possible to characterize the functional relationship between $e(S_{max})$ and moisture, therefore fitted lines or curves are omitted.

At the intermediate moisture content (10 to 14% d.b.), which falls within the typical range for the NIR hardness and SKCS hardness methods) (table 1), the relationship between S_{max} and NIR hardness or S_{max} and SKCS hardness was positively correlated (fig. 8). Based on all 10 NIST samples, S_{max} demonstrated a slightly higher correlation with NIR hardness than with SKCS hardness (r^2 = 0.84 vs 0.77); however, with removal of Hard No. 4, the correlation between S_{max} and SKCS hardness ($r^2 = 0.97$) was higher than that between any other physical strength property and NIR or SKCS hardness, with or without inclusion of this sample. Hard No. 4 exhibited the highest compressive strength, yet its hardness value by NIR or SKCS was substantially less than that of Hard No. 5. Coefficients of determination for the other non-strain properties related to NIR hardness are as follows: E: r^2 = 0.85 (0.86 without Hard No. 4), $W(S_{max})$: r^2 = 0.74 (0.85). Likewise, for SKCS hardness, r^2 values were as follows: E: r^2 = 0.81 (0.90), $W(S_{max})$: r^2 = 0.62 (0.86). While these

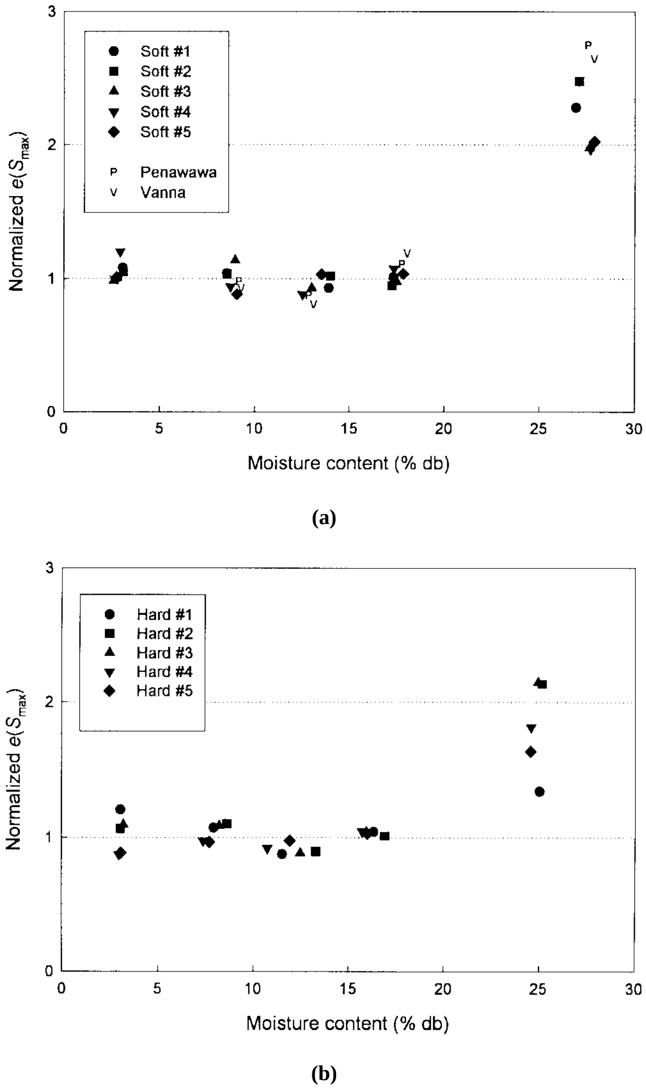


Figure 7–Effect of moisture content on strain at point of maximum compressive stress [e(S_{max})]. (See fig. 4 caption for graph descriptions.)

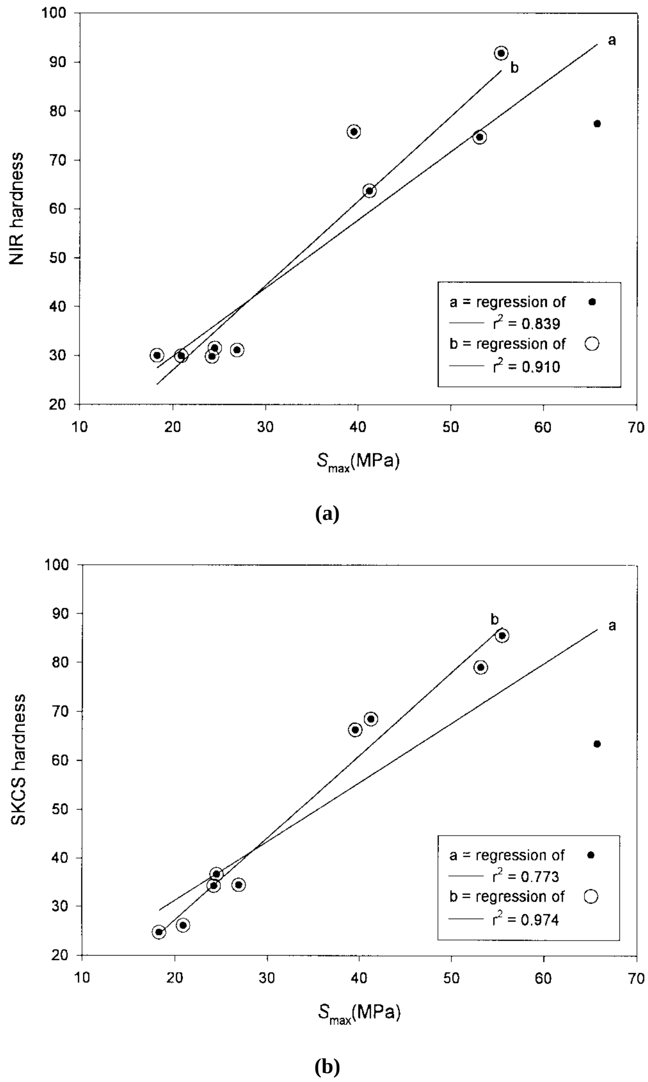


Figure 8–Relationships between working definitions for wheat hardness and S_{max}, as measured on NIST samples [a = Near-infrared (NIR) hardness; b = Single kernel characterization system (SKCS) hardness].

correlations suggest that SKCS hardness may be a more accurate measurement of wheat physical compressive strength than NIR hardness, additional testing with new samples is warranted.

Discussion

Wheat hardness is generally believed to arise from the interaction between starch granules and endosperm proteins (Barlow et al., 1973). The presence of a 15 kDa protein, termed friabilin (Greenwell and Schofield, 1986) and later postulated to be a family of related proteins (Morris et al., 1994), results in a softening of wheat texture through a weaker bond between the endosperm matrix, the starch granule, and certain bound polar lipids (Greenblatt et al., 1995). The extent of this bond does not seem to become apparent until the action of cellular desiccation that immediately precedes grain maturity (Bechtel et al., 1996). In fact, Bechtel et al. (1996) found that when immature wheat kernels were freeze-dried, kernel hardness was substantially less than if kernels of the same state of maturity were allowed to air-dry. This suggests that wheat texture is brought on by a final mixing of cellular components during drying and an eventual binding of specific cellular components to the starch granule surface. Even when mature wheat is milled, fractionated into starch and gluten, then reconstituted in the presence of water and eventually dried, texture, as defined by tensile strength of pressed flour tablets, is inherent to the original stock; that is, tensile stress is greater in hard wheats than soft wheats (Malouf and Hoseney, 1992). Based on fracture mechanics theory and experimental measurements of compressive strength during loading/unloading cycles and force of cutting during microtoming, Dobraszczyk (1994) has since shown that the starch-protein matrix is largely dependent on the degree of vitreousness in the kernel, with vitreous kernels exhibiting more than twice the fracture toughness as mealy kernels of the same variety. Irrespective of the degree of vitreousness, fracture toughness decreases as the moisture content increases (Dobraszczyk, 1994). The present study has demonstrated the degree to which moisture affects wheat texture. It shows that soft and hard wheats exhibit the same trend with moisture content; however, they do so at different response rates. Similar to the findings of Glenn et al. (1991), the present study has found that moisture affects endosperm compressive strength of hard wheat more greatly than soft wheat. Additionally, hard wheat samples generally have a more linear response between S_{max} and moisture content than soft wheat samples. Although less linearly related to moisture content than S_{max}, the response of E to moisture content is shown to be more universal, with only a small difference in slope noted between hard and soft wheats.

According to Pomeranz and Williams (1990) the inverse relationship between hardness and moisture content arises from a cleaving action of water as it diffuses into the intermicellar spaces of the kernel, causing swelling, a weakening of the cohesion forces, and in particular, a lowering of the crushing resistance of the endosperm. The negative correlation between moisture content and S_{max}, E, or $W(S_{max})$ is intuitively logical, yet in contrast with that reported for NIR hardness on hard wheats (Norris et al., 1989). This effect of moisture on NIR hardness was also noted for soft wheats (Windham et al., 1993) and later explained to arise from an increased moisture content causing larger size pericarp particles to occur during grinding (Gaines and Windham, 1998). Because the two wavelengths used in NIR hardness calculation (1680 and 2250 nm) were purposely selected for their sensitivity to particle size (with larger particles causing higher apparent absorption), any event that causes a shift in the particle size distribution to larger size will consequently result in a higher NIR hardness value. A means to compensate the NIR hardness value for moisture content deviation is available (Windham et al., 1993; AACC, 1995). The purpose of comparing past NIR studies with the present study is the ability to highlight the differences in the bases of NIR hardness and physical strength properties. Gaines and Windham (1998) remark on the illogical, wetter-being-harder, behavior of moisture content and wheat hardness as measured by NIR. They further demonstrate that when moisture change occurs after (rather than before) milling, NIR hardness value is unchanged, which emphasizes the importance of particle size on NIR hardness. Therefore, the manner in which wheat texture is defined has a direct bearing on how it describes moisture tempering. At a fixed moisture content such as that for safe ambient storage, NIR wheat hardness and physical strength properties of wheat endosperm are directly related to each other. However, the difference in the basis of measurement (particle size vs. compressive strength) causes different responses to moisture content variation. Although SKCS data on moisture content variation is currently unavailable, it is believed that the behavior of moisture content on SKCS hardness is similar to that for the physical strength properties [S_{max}, E, and $W(S_{max})$], due to their closely related bases of measurement. In a recent comparison of wheat hardness measurement methods using a set of chromosome 5D homozygous recombinant substitution lines derived from a soft wheat cultivar and a hard wheat cultivar, Morris et al. (1999) found that the SKCS procedure is a better measurement of hardness [as manifested by a major gene located on the short arm of chromosome 5D (Mattern et al., 1973)] than the methods of NIR, particle size indexing, or laboratory-scale milling.

In summary, the moistening of wheat kernels from a level considered safe for storage to one that is suitable for milling results in a weakening of the endosperm. For example, an increase in moisture content of 10 percentage units (d.b.) above typical ambient storage conditions results in a 35 and 55% reduction of maximum compressive stress for soft and hard wheats, respectively. The slope of the stress-strain curve becomes shallower with increased moisture and the point of mechanical failure becomes poorly defined. The effect of moisture on compressive strength properties is greater for hard wheats than for soft wheats. This difference is most apparent in measurements of S_{max} and $W(S_{max})$, for which hard wheat is, respectively, 56% and 230% more affected by moisture change than soft wheat. The results of this study will form a basis for the future understanding of how single-kernel hardness instruments, such as the SKCS, react to change in kernel moisture.

ACKNOWLEDGMENTS. J. D. Shaffer (ARS, Beltsville) prepared and tested wheat cylinders. B. T. Vinyard (ARS, Beltsville) provided guidance in performing the analyses of covariance. C. F. Morris (Pullman, Wash.) supplied non-NIST wheat samples. This research was supported in part by USDA NRICGP grant 95-37500-1927.

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